Supporting Information

Quantifying Thiol Ligand Density of Self-assembled Monolayers on Gold Nanoparticles by Inductively Coupled Plasma Mass Spectrometry

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1. ICPMS MEASUREMENTS

1.1. DETERMINATION OF MATRIX OF CHOICE FOR ICPMS MEASUREMENTS

For preliminary investigations and method optimization GNPs of different sizes were diluted 1:10 using H_2O, 2% (v/v) HCl and 2% (v/v) HNO_3, respectively. All solutions were purified before use by sub-boiling distillation. Aim of the preceding study was to investigate the influence of different matrices on the analysis results, evaluated by means of the measurement precision. The precisions of the measured intensities of the analytes of interest are given in Table S1. It can be seen that measurements performed in a 2% (v/v) HCl matrix yielded better precisions for the measured intensities compared to other GNP matrix solutions (*i.e*. H_2O and HNO_3). Thus, 2% (v/v) HCl was the matrix of choice for the subsequent GNP analyses.

Table S1: Precision of measured intensities for GNPs of different sizes coated with 16-mercaptohexadecanoic acid (MHA)

Sample	Matrix	Measured intensity RSD / %			
		$^{32}S^{16}O$	$^{34}S^{16}O$	^{197}Au	^{115}In
GNP (10 nm)	H_2O	2.4	3.2	1.6	7.5
	2 % (v/v) HNO_3	4	4.2	2.8	2
	2 % (v/v) HCl	**2.2**	**4.2**	**2**	**0.8**
GNP (25 nm)	H_2O	3.9	3.2	4.4	7.1
	2 % (v/v) HNO_3	10.9	8.7	12.4	7.3
	2 % (v/v) HCl	**2.1**	**3.3**	**1.9**	**1**
GNP (50 nm)	H_2O	6	5.1	3.3	10.2
	2 % (v/v) HNO_3	4.1	3.5	3.1	1.1
	2 % (v/v) HCl	**3.2**	**5.1**	**2.2**	**1.1**

In a next step, the influence of prior dissolving of Au in aqua regia compared to dilution with 2% (v/v) HCl as matrix before GNP analysis on the ^{197}Au / $^{32}S^{16}O$ ratio was examined. Thereby, no significant difference between the two matrices could be

observed with both between 7-10% RSU (k=2) (data not shown). This is also in good accordance with Allabashi et al. (*J.Nanopart.Res.* 2009, *11*, 2003-2011), who observed that a preliminary digestion of GNP colloid solutions with aqua regia is not always necessary.

1.2. Uncertainty calculation

Expanded (k=2) total combined standard uncertainties (U_c) were calculated according to ISO/GUM (ISO/IEC Guide 98-3, 2008) and EURACHEM (EURACHEM/CITAC Guide CG 4) guidelines. The single parameters propagated for computing the expanded total combined standard uncertainties of the Au/S measurements are given in Table S2.

Table S2: Propagated parameters for computing the expanded total combined standard uncertainties

Propagated parameter
Slope (k) of linear regression - S
Slope (k) of linear regression - Au
Intercept (d) of linear regression - S
Intercept (d) of linear regression - Au
Intensity (I) of S
Intensity (I) of Au
Intensity (I) of In

The model equation used for calculation of U_c is given in eq. S1.

(eq S1)
$$\frac{Au}{S} = \frac{\left(\frac{\left(\frac{I_{Sample(Au)}}{I_{Sample(In)}} - \frac{I_{Blank(Au)}}{I_{Blank(In)}}\right) - d_{Au}}{k_{Au}}\right) \times 10^{-9}}{196.97} \Bigg/ \frac{\left(\frac{\left(\frac{I_{Sample(S)}}{I_{Sample(In)}} - \frac{I_{Blank(S)}}{I_{Blank(In)}}\right) - d_{Au}}{k_{S}}\right) \times 10^{-9}}{32.06}$$

1.3. Spiking recovery experiments

Au standards (10 ppm) as well as citrate stabilized GNP solutions were spiked with S standards to evaluate the influence of the presence of Au on sulfur measurements.

Within the working range of sulfur measurements no detrimental effect of gold on the recovery measurements of sulfur was found and also in the high concentration area of sulfur concentrations the recovery is still within RSU of 10%.

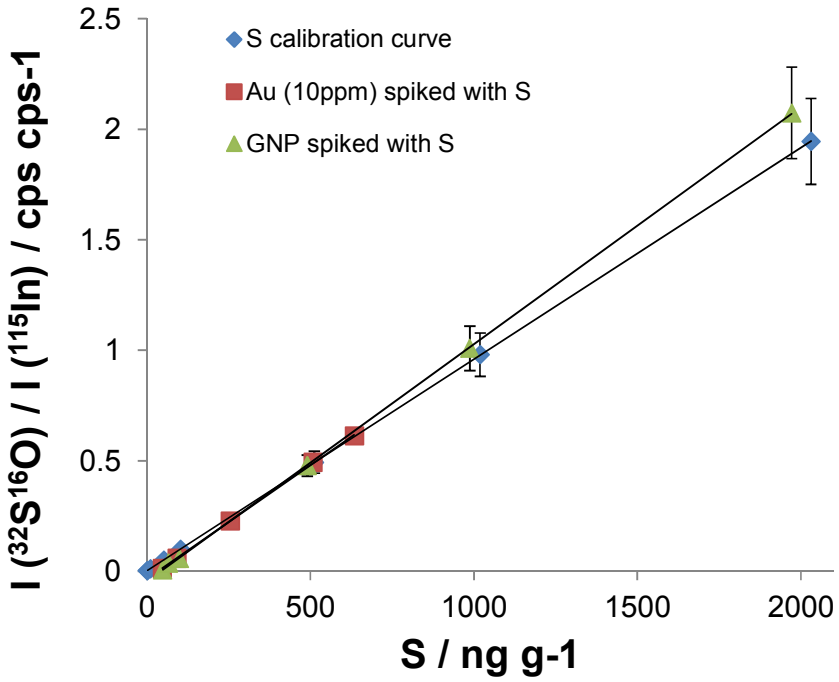

Figure S1: Validation of recovery of sulfur in presence of gold.

2. DETERMINATION OF LIGAND DENSITY

Figure S2 shows the dependencies of the Au/S ratio on the GNP size for the distinct nanoparticle types, *i.e*. GNPs modified with different ligands. The corresponding linear regression data for each type of particles are given in Table 2 of the main document.

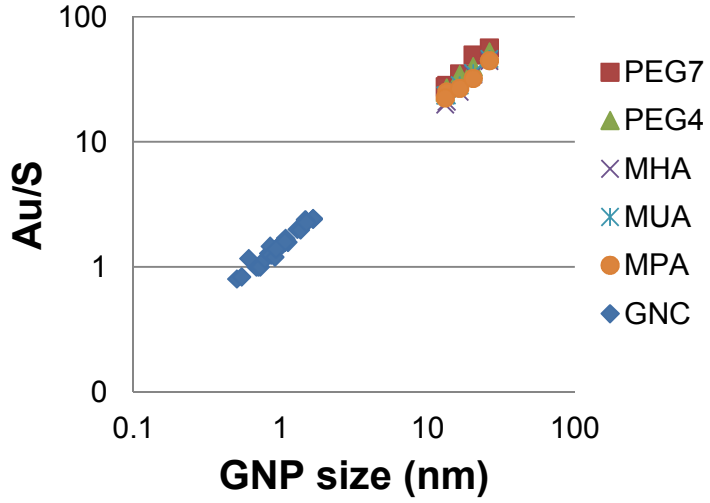

Figure S2: Plot of Au/S ratio as determined by ICPMS measurement vs. GNP size and comparison to data from literature for gold nanoclusters (GNC) measured by X-ray crystallography and density functional theory (DFT) studies.

3. CALCULATIONS OF LIGANDS BOUND PER GNP

Besides surface coverage, also the totally available surface and the concentration of GNPs must be known for the calculation of the totally available ligand concentration bound onto GNPs. Along this line, the concentration of GNPs can be calculated applying the following consideration. The number of nanoparticles N_{GNP} in solution arise from the ratio of the initial Au(III) atoms $N_{Au(III)}$ (*e.g*. for this study 55 mL of 1.14 mM $HAuCl_4$ final concentration were used) to the number of gold atoms per GNP $N_{Au/GNP}$. At a citrate/$HAuCl_4$ ratio > 1.5 the conversion of Au(III) to colloidal gold is quantitative and hence it follows (eq.S2)

(eq.S2) $$N_{GNP} = \frac{N_{Au(III)}}{N_{Au/GNP}} = \frac{1.14E-03M \times 0.055L \times N_A}{\frac{\pi}{6}\frac{\rho D^3}{M_{Au}}} = 1.22E18D^{-3}$$

The molar concentration of nanoparticles c_{GNP} in solution can be calculated by (eq.S3)

(eq.S3) $$c_{GNP} = \frac{N_{GNP}}{VN_A} = 3.69E-05D^{-3}$$

wherein N_A is Avogadro´s constant and V the volume of the solution.

In practice, the totally available ligand concentration c_L per colloidal NP suspension c_{GNP} but not the ligand density itself is of practical experimental relevance in many applications of functionalized GNPs (*e.g.* in adsorption studies). It can be calculated for the different GNP sizes and ligand lengths taking into account the determined surface coverages for individual modified GNPs and the GNP concentrations in solutions. Complete conversion of Au(III) which has been proven for C/H ratios > 1.5 and no loss of thiol ligand after washing is assumed. The results are summarized in Table S3. It can be seen that the molar ligand concentration drops by a factor of about 2 when a PEG_7-spacer is used instead of a $-(CH_2)_3$-spacer such as in MPA modified GNPs. On the other hand, the ligand concentration is by a factor of 4 decreased when 13 nm particle size is exchanged for 26 nm particle diameter. This clearly emphasizes the overall benefit of smaller nanoparticles in adsorption based applications.

Table S3: Calculated ligand concentrations for GNPs of different sizes prepared by variation of the citrate/$HAuCl_4$ (C/H) ratio. Values were calculated for initial $HAuCl_4$ concentration of 1.14 mM and volume of 55 mL.

C / H	GNP Size, (TEM, nm)	MW (GNP)	c , GNP (mM)	Surface / GNP (nm^2)	surface area (m^2 ml^{-1})	mM ligand/mM GNP				
						MPA	MUA	MHA	PEG4	PEG7
2	26.2	1.10E+08	2.0E-06	2164	2.66E-03	1.84E+07	1.23E+07	9.95E+06	1.08E+07	9.30E+06
3	20.4	5.14E+07	4.4E-06	1303	3.43E-03	1.11E+07	7.42E+06	5.99E+06	6.51E+06	5.60E+06
4	16.5	2.73E+07	8.2E-06	854	4.23E-03	7.26E+06	4.87E+06	3.93E+06	4.27E+06	3.67E+06
5	13.2	1.39E+07	1.6E-05	544	5.30E-03	4.63E+06	3.10E+06	2.50E+06	2.72E+06	2.34E+06
6	13.5	1.49E+07	1.5E-05	571	5.18E-03	4.86E+06	3.26E+06	2.63E+06	2.86E+06	2.46E+06

4. UV/VIS SPECTRA OF GNPS

Citrate stabilized GNPs were characterized by measuring their surface plasmon resonance (SPR) spectra. The corresponding spectra are shown in Figure S3.

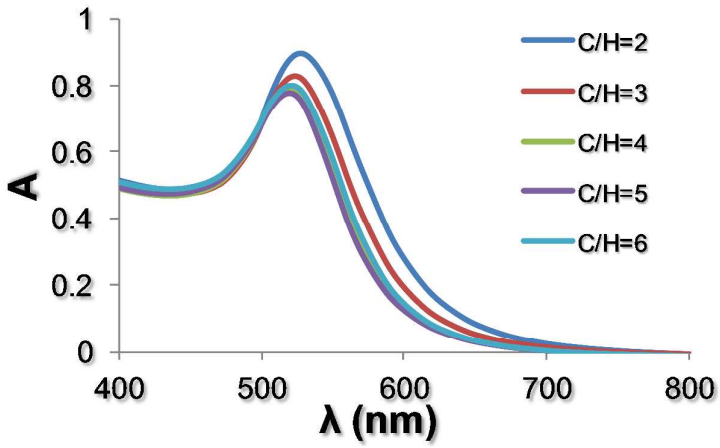

Figure S3: SPR spectra of citrate stabilized GNPs